Journal of Molecular Catalysis B: Enzymatic 103 (2014) 41–46

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Phenol oxidation by DyP-type peroxidases in comparison to fungal and plant peroxidases

Christiane Liers[a,*], Elizabet Aranda[a,1], Eric Strittmatter[b], Klaus Piontek[b], Dietmar A. Plattner[b], Holger Zorn[c], René Ullrich[a], Martin Hofrichter[a]

[a] TU Dresden, International Institute Zittau, Markt 23, 02763 Zittau, Germany
[b] University of Freiburg, Institute of Organic Chemistry, Albertstrasse 21, 79104 Freiburg, Germany
[c] Justus-Liebig-University Giessen, Heinrich-Buff-Ring 58, 35392 Gießen, Germany

ARTICLE INFO

Article history:
Available online 7 October 2013

Keywords:
Dye-decolorizing peroxidases
Compound II/resting state
Redox potential

ABSTRACT

Over the last years, novel peroxidases secreted by lignocellulose-degrading agaricomycetes have been discovered. Among them, the so-called DyP-type peroxidases (DyPs) that are secreted under conditions close to nature (i.e. in wood cultures) are of particular interest, since they are able to oxidize diverse substrates including veratryl alcohol, non-phenolic lignin model dimers as well as recalcitrant phenols and dyes. In spite of their unique protein structure and their catalytic versatility, the estimation of the redox potential of this new peroxidase group is still pending. To solve this problem, we used a catalytic approach developed by Ayala et al., 2007 [21], which is based on the Marcus equation and the determination of the redox thermodynamics between heme-peroxidase compound II and the resting state enzyme. Five fungal DyPs (among them four wild-type enzymes and one recombinant protein) were tested regarding phenol oxidation in comparison to other well-studied plant and fungal peroxidases (soybean peroxidase, SBP, *Coprinopsis cinerea* peroxidase, CiP, lignin peroxidase of *Phanerochaete chrysosporium*, LiP). DyP-type peroxidases have a high affinity for phenols and can oxidize even recalcitrant representatives such as *p*-nitrophenol. Based on this "phenol oxidation method", their redox potential was estimated to range between 1.10 ± 0.02 and 1.20 ± 0.1 V, which is between the values calculated for high-redox potential LiP (1.26 ± 0.17 V) and low-redox potential, phenol-oxidizing plant (0.93 ± 0.04 V for SBP) and fungal (1.06 ± 0.07 V for CiP) peroxidases.

1. Introduction

Heme-containing peroxidases (donor: H_2O_2 oxidoreductases, EC 1.11.x) are versatile catalysts oxidizing diverse substrates. They are ubiquitous in fungi, plants, animals and eubacteria. On the basis of sequence homologies, they are classified within different superfamilies, among which those of animal and non-animal peroxidases (former plant peroxidases) are the largest groups (PeroxiBase; http://peroxibase.toulouse.inra.fr/index.php). Non-animal peroxidases comprise three classes: class I – intracellular, organelle-associated and bacterial peroxidases (e.g. cytochrome c peroxidase), class III – secreted plant peroxidases (e.g. horseradish peroxidase, HRP) and class II – secretory fungal peroxidases including ligninolytic peroxidases [1]. The latter are exclusively found in wood and leaf-litter decomposing Basidiomycotina (mostly agaricomycetes) and comprise manganese, lignin and versatile peroxidases (MnP, LiP and VP). They possess high redox potentials (1.1–1.5 V), which enable them to oxidize a huge number of recalcitrant substrates including methoxylated aromatics present in wood and other lignocelluloses [2,3].

Two new superfamilies of fungal peroxidases were discovered over the last decade, namely unspecific peroxygenases and dye-decolorizing peroxidases (DyPs). The enzymes of the latter superfamily are, according to genetic databases, widespread not only among agaricomycetes but also among bacteria[2] and

Abbreviations: DyPs, DyP-type peroxidases; *Aau*DyP, *Auricularia auricula-judae*; *Egl*DyP, *Exidia glandulosa*; *Mep*DyP, *Mycena epipterygia*; *Msc*DyP, *Mycetinis scorodonius* (formerly *Marasmius scorodonius*); CiP, *Coprinopsis cinereus* peroxidase; LiP, lignin peroxidase; MnP, manganese peroxidase; VP, versatile peroxidase; SBP, soybean peroxidase; HRP, horseradish peroxidase; TOP, tobacco peroxidase.

* Corresponding author. Tel.: +49 3583 612754; fax: +49 3583 612734.
E-mail address: liers@ihi-zittau.de (C. Liers).
[1] Present address: Department of Soil Microbiology and Symbiotic Systems, Estación Experimental del Zaidín, CSIC, Professor Albareda n°1, 18008 Granada, Spain.

[2] It has to be noted that bacterial and fungal DyPs share hardly sequence homology (<5%), except for a few key residues [5] so that an old age or a convergence evolution can be assumed [4].

1381-1177
http://dx.doi.org/10.1016/j.molcatb.2013.09.025

ascomyceteous fungi [4,5]. DyPs possess an "atypical" molecular architecture and divergent mechanistic behavior (that is not fully understood yet [4]), which does not allow their classification within the class II peroxidases [5–7]. Despite the structural differences, the physico-chemical properties of DyPs resemble those of classic heme proteins, e.g. regarding UV–vis spectral characteristics, molecular masses or isoelectric points [5,8,9].

Meanwhile twelve DyPs – eight fungal and four bacterial proteins – have been characterized [5,6]. We have recently found that two DyP isoforms of the jelly fungus *Auricularia auricula-judae* (*Aau*DyP1 and 2) can oxidize non-phenolic aromatics (e.g. veratryl alcohol and a β-*O*-4 lignin model dimer) [6], a catalytic property that is actually characteristic for high-redox potential peroxidases (LiP, VP) [10–12]. Since these reactions showed just moderate catalytic efficiency at pH 3 and very low pH optima (pH < 2), where it is easier to abstract single electrons from aromatic rings [13,14], we concluded that the redox potential of DyPs may range between those of ligninolytic peroxidases (e.g. ~1.5 V for LiP H8 calculated via oxidation of methoxybenzenes; [15]) and low-redox potential plant peroxidases (e.g. ~0.8 V for HRP; [13,16]).

The catalytic cycle of heme peroxidases is initiated by a rapid two electron transfer from the native ferric heme protein ($PorFe^{III}$) to H_2O_2, to give a higher oxidation state of the enzyme called compound I ($^{\bullet+}PorFe^{IV}=O$). It is a ferryl π–cation radical with a formal oxidation state of +5 for the iron (Fe^{V}) and oxygen coupled to this iron by a double bond (Eq. (1); [17]). In the peroxidative cycle, compound I is reduced by two consecutive one-electron reductions via compound II ($PorFe^{IV}=O$) to the native ferric enzyme (Eq. (2)). In dependence of the redox potentials of compound I and II, numerous substrates can serve as electron donors in these reactions, e.g. phenolic and non-phenolic aromatics, metal ions or complex dye-stuff molecules [5].

$$PorFe^{III} + H_2O_2 \rightarrow {}^{\bullet+}PorFe^{IV} = O + H_2O \quad (1)$$

$$^{\bullet+}PorFe^{IV} = O + 2e^- + 2H^+ \xrightarrow{k_{ET}} PorFe^{III} + H_2O \quad (2)$$

$$H_2O_2 + 2e^- + 2H^+ \rightarrow 2H_2O \quad (3)$$

There are different methods for the direct estimation of the redox potential of oxidative enzymes (redox couple compound I/native enzyme) like rapid kinetic techniques (e.g. stopped-flow kinetics, voltammetric methods; [18–20]) but also indirect methods based on the spectral determination of the equilibrium between high oxidation state species of peroxidases have been developed [16,21].

The Marcus theory of outer-sphere electron transfer expects a semi logarithmic dependence between the rate of an electron transfer reaction (e.g. k_{ET}) and a thermodynamic driving force [22]. This correlation can also be considered between the specific activity of a peroxidase-catalyzed reaction and the ionization energy of the substrate, which has successfully been used for the calculation of the redox potentials of some plant and fungal oxidoreductases (e.g. laccase, HRP and VP) [21,23–26]. It was postulated that the observed initial rate for the oxidation of a one-electron donor substrate (e.g. k_{cat}) reflects the electron transfer from the substrate to the enzyme (i.e. k_{ET}). Under saturated and optimized reaction conditions (e.g. by using small polar substrates such as phenol derivatives), this correlation can be used for the estimation of the standard reduction potential. Thus, kinetic data obtained from the initial oxidation rates of *p*-substituted phenols were normalized with the help of the maximum activities obtained for a number of peroxidases and extrapolated against the substrates' redox potential [21,27].

Our report describes for the first time the calculation of the redox potential of several DyP-type peroxidases by a simple and fast method based on the Marcus theory [22], which has been adapted from Ayala et al. [21]. To this end, five of the eight so far characterized fungal DyPs were studied with respect to the oxidation of different phenolic substrates.

2. Materials and methods

2.1. Organisms and enzyme production

All fungi used for the production of wild-type DyPs are deposited in the German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig) or in the culture collections of the Department Food and Environmental Sciences (University of Helsinki, Finland) and the Department of Environmental Biotechnology (International Institute Zittau, Germany). The fungi were stored on 2% malt-extract agar (MEA) in culture slants at 4 °C in the dark and routinely pre-cultured on 2% MEA plates for 2 weeks.

Wild-type DyPs of *Exidia glandulosa* (*Egl*DyP), *Mycena epipterygia* (*Mep*DyP) and *Auricularia auricula-judae* (*Aau*DyP 1 and 2) were produced and purified as described recently [8,28]. Recombinant DyP of *Mycetinis scorodonius* (r*Msc*DyP) was provided by DSM (Delft, The Netherlands) [28]. For comparison, *Coprinopsis (Coprinus) cinerea* peroxidase (CiP) was produced according to Ikehata et al. [29] in a glucose–peptone–malt extract medium. Soybean peroxidase (SBP) was purchased from Sigma–Aldrich (Weinheim, Germany). Both enzymes were subjected to purification by FPLC and the final step was carried out on an anion exchanger (MonoQ, 10 mM sodium acetate buffer; pH range 4.0–5.5, gradient 0 to 0.3 or 1.0 M NaCl) as described in [8]. Crude LiP from *Phanerochaete chrysosporium* was purchased from JenaBios GmbH (Jena, Germany) and purified as described recently [28]. The purity of all used peroxidases was proved by SDS-PAGE and by determination of the Reinheitszahl ($RZ = A_{407}/A_{280}$) [8,28].

2.2. Enzymatic reactions

Activity of DyPs was routinely measured with ABTS as described previously [8]. The pH optima for the oxidation of phenolic substrates were determined using 2,6-dimethoxyphenol (2,6-DMP) instead of ABTS as the substrate; the pH of the sodium citrate buffer (50 mM) was varied in the range from 3.0 to 6.0. In case of SBP and CiP, the enzyme activity was determined accordingly but at their own optima of pH 5.5 and 6.8, respectively [21,30]. LiP activity was determined with veratryl alcohol as described elsewhere [31].

The following *p*-substituted phenols with increasing redox potentials were used for the oxidation studies: *p*-methoxyphenol, *p*-chlorophenol, *p*-bromophenol, phenol, *p*-hydroxyacetophenone, *p*-hydroxybenzoic acid, *p*-hydroxybenzonitrile as well as *p*-nitrophenol [21]. All phenols were purchased from Sigma–Aldrich with the highest purity available. Organic solvents and hydrogen peroxide (H_2O_2, 30%) were obtained from Merck (Darmstadt, Germany).

In-vitro reactions were carried out in 0.5 ml reaction tubes (Eppendorf, Hamburg, Germany) in a total volume of 250 μl and under slightly modified conditions as described by Ayala et al. [21]. Compared to the latter report, a lower concentration of hydrogen peroxide (0.1 mM) was applied in order to avoid protein damaging and heme-bleaching effects [32]. Reactions were started by the addition of H_2O_2 and kept under permanent stirring at a constant temperature of 23 °C. They were stopped by addition of trifluoroacetic acid (final concentration 2%) and the phenol derivate concentration was detected by HPLC. Preliminary experiments for each enzyme were performed to verify the kinetics of the enzymatic reactions. Every 10 s, samples were collected and analyzed over a total reaction time of 40 s. After evaluation of these data, a reaction time of 10 s was ascertained to be optimal (Fig. 1A).

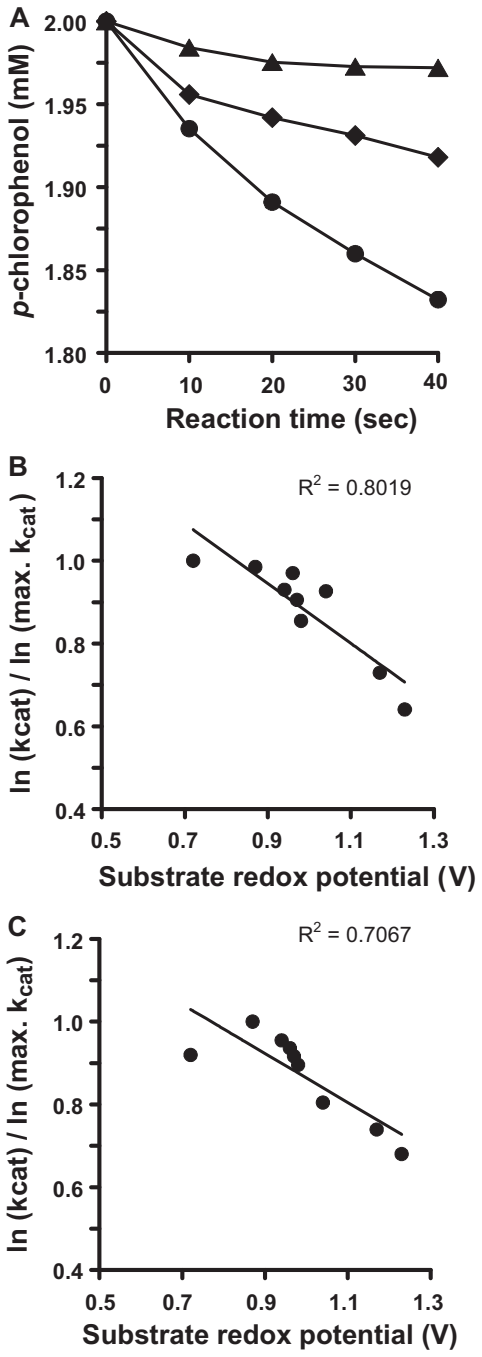

Fig. 1. Time course of the oxidation of *p*-chlorophenol (A) by 73 nM r*Msc*DyP (circles), 24 nM *Aau*DyP2 (squares) and 3 nM SBP (triangles) in the presence of 100 μM H_2O_2. Semi-logarithmic plots of the normalized turnover numbers (k_{cat}) for the oxidation of *p*-substituted phenols versus the redox potential of the substrates by the example of *Aau*DyP2 (B) and *P. chrysosporium* LiP (C).

The different *p*-substituted substrates were applied in peroxidase-saturating final concentrations of 2 mM and the appropriate reaction buffer was used for each peroxidase (50 mM final concentration; LiP: sodium tartrate pH 3.0; DyPs: sodium acetate pH 4.5; SBP: sodium acetate pH 5.5; CiP: citrate/phosphate pH 6.8) similar as described by Ayala et al. [21]. Oxidation of the phenolic substrates was followed by HPLC using a reversed phase column (C18-column, Synergi, Phenomenex®, Aschaffenburg, Germany) and an HPLC system HP 1200 (Agilent®, Waldbronn, Germany) equipped with a diode array detector (DAD; 190–700 nm). Elution was performed under isocratic conditions using appropriate water–acetonitrile mixtures as mobile phase [21]. All reactions were performed in triplicate.

2.3. Homology modeling

To verify a possible long range electron transfer (LRET) in the tested DyPs homology models of *Egl*DyP, *Mep*DyP, and *Msc*DyP were built using the Swiss-Model Server [33] and the software Modeller [34], respectively. The structure of *Aau*DyP (pdb code 4au9) was chosen as template model. Electron-transfer-pathway calculations were performed as previously described [7]. Images were created in PyMOL [35].

3. Results and discussion

To find indications for the biochemical function and possible physiological role of the recently classified new (super)-family of DyP-type peroxidases [5,36], it is of general importance to estimate their redox potential. In this report, we provide first data on the redox thermodynamics between the last substrate-oxidizing DyP-intermediate (probably a compound II-like reactive enzyme state) and the native enzyme (ferric DyP). To this end, we studied the oxidation of a series of phenolic substrates by a representative selection of fungal DyPs, five wild-type enzymes and one recombinant one.

The five DyPs oxidized all phenolics tested here including the high-redox potential compounds (Table 1). Thus *p*-nitrophenol, the substrate with the highest ionization potential (around 1.2 V, [37,38]), was converted with turnover numbers (k_{cat}) between 88 and 435 min^{-1}. In fact, there are only a few reports on the *in vitro* oxidation of *p*-nitrophenol by fungal heme-peroxidases (e.g. LiP and MnP) [39]. In the study of Ayala et al. (2007), VP, lactoperoxidase (LPO) and CiP were found to convert *p*-nitrophenol with low turnover numbers between 10 and 25 min^{-1} whilst plant peroxidases (SBP, HRP) were not able to oxidize this recalcitrant phenol [21]. In our study, the latter finding was confirmed but fungal CiP showed a quite high turnover number for *p*-nitrophenol (343 min^{-1}) that is about 20-fold higher than that reported by Ayala et al. (k_{cat} = 18 min^{-1}) (Table 1, [21]). This could be explained by the modified reaction time that was 10 min in case of [21] but just 10 s in our study. To obtain apparent kinetic data of peroxidases suitable for further calculations, the reaction time used should be always taken with care, since the velocity tends to decline in the course of reaction and can lead to an underestimation of the initial activity [40,41]. In a set of preliminary experiments, we have demonstrated that the linear course of oxidation (e.g. of *p*-chlorophenol) is relatively short and already declined after 10 s of reaction (Fig. 1A). Therefore, we used a reaction time of 10 s for all calculations to ensure the linearity of oxidation rates, which were then normalized with the maximum activity value and plotted against the substrate redox potential [27]. An example for the linear correlation between the initial oxidation rate (k_{cat}) of the electron donor substrate and the ionization energy of the phenolic substrate is given for *Aau*DyP1 and LiP in Fig. 1B and C, respectively.

Unlike LiP and MnP, DyPs do not require a redox-mediator (aryl cation radical of veratryl alcohol and Mn^{3+}, respectively) to accomplish the oxidation of phenolic substrates. They resemble in this context rather VP that shows a strong phenol oxidizing activity independent of any redox mediator [42]. Furthermore, in a recent study, we have already described the direct oxidation of non-phenolic aromatics (e.g. veratryl alcohol) by *Aau*DyP in a VP/LiP-like reaction [28]; (such compounds can be oxidized by MnP merely in the presence of additional redox mediators, e.g. thiols or unsaturated fatty acids [43,44]). On the other hand, DyPs clearly differ from VP in their inability to oxidize manganese ions

Table 1
Turnover number (k_{cat}) of fungal and plant peroxidases for the oxidation of *p*-substituted phenols with different redox potential.

Phenol	Redox potential (V)	k_{cat} (min^{-1})[a]							
		*Pch*LiP	r*Msc*DyP	*Aau*DyP I	*Aau*DyP II	*Mep*DyP	*Egl*DyP	CiP	SBP
RZ		1.4	0.6	1.0	1.7	0.8	1.0	2.0	1.3
p-Methoxyphenol	0.72	2813	3481	7604	13,092	4005	20,433	153,184	18,104
p-Methylphenol	0.87	5.630	2801	8979	11,372	7168	17,431	70,300	32,978
p-Chlorophenol	0.94	3808	1888	5084	6750	2788	8244	85,506	3391
p-Bromophenol	0.96	3241	3001	7344	9882	4496	11,905	144,585	10,673
Phenol	0.97	2734	1265	4123	5356	3168	9401	58,808	917
p-Hydroxyacetophenone	0.98	2285	1711	2918	3307	1979	4473	57,608	375
p-Hydroxybenzoic acid	1.04	1042	1234	4976	6529	2642	8476	15,143	109
p-Hydroxybenzonitrile	1.17	593	508	954	1008	933	1587	15,143	0
p-Nitrophenol	1.23	356	88	306	435	152	165	343	0

[a] Molecular weights (kDa) used for calculations: *Pch*LiP – 42, r*Msc*DyP – 69, *Mep*DyP – 61, *Egl*DyP – 56, *Aau*DyP I – 51, *Aau*DyP II – 43, CiP – 40, SBP – 47.

(Mn^{2+}) [28,42]. LiP – though showing a high affinity to phenols and a high initial oxidation rate – can poorly oxidize them, since it is rapidly inactivated [41,45]. This inactivation of LiP was proposed to be mainly due to the accumulation of compound III, which cannot be reverted to the ferric enzyme by phenols or phenoxyl radicals [31]; it can be prevented in the presence of veratryl alcohol that may act as a redox mediator [45].

Assuming that the redox potential of compound I/compound II and compound II/resting state are similar (e.g. as demonstrated for lacto- and myeloperoxidases [46]), the oxidative threshold resulting from this correlation can be supposed as the redox potential of the couple compound II/resting state [16,21,46]. The values determined for the reference enzymes SBP and CiP (0.93 and 1.06 V, respectively; Table 2) are comparable to those described by different authors who used both spectral (0.9 and 1.04 V, respectively) and direct catalytic methods (0.95 and 1.18 V, respectively) [16,21,46]. In general, similar experimental data have also been reported for other peroxidases such HRP (0.87 and 0.89 V, [16,21])

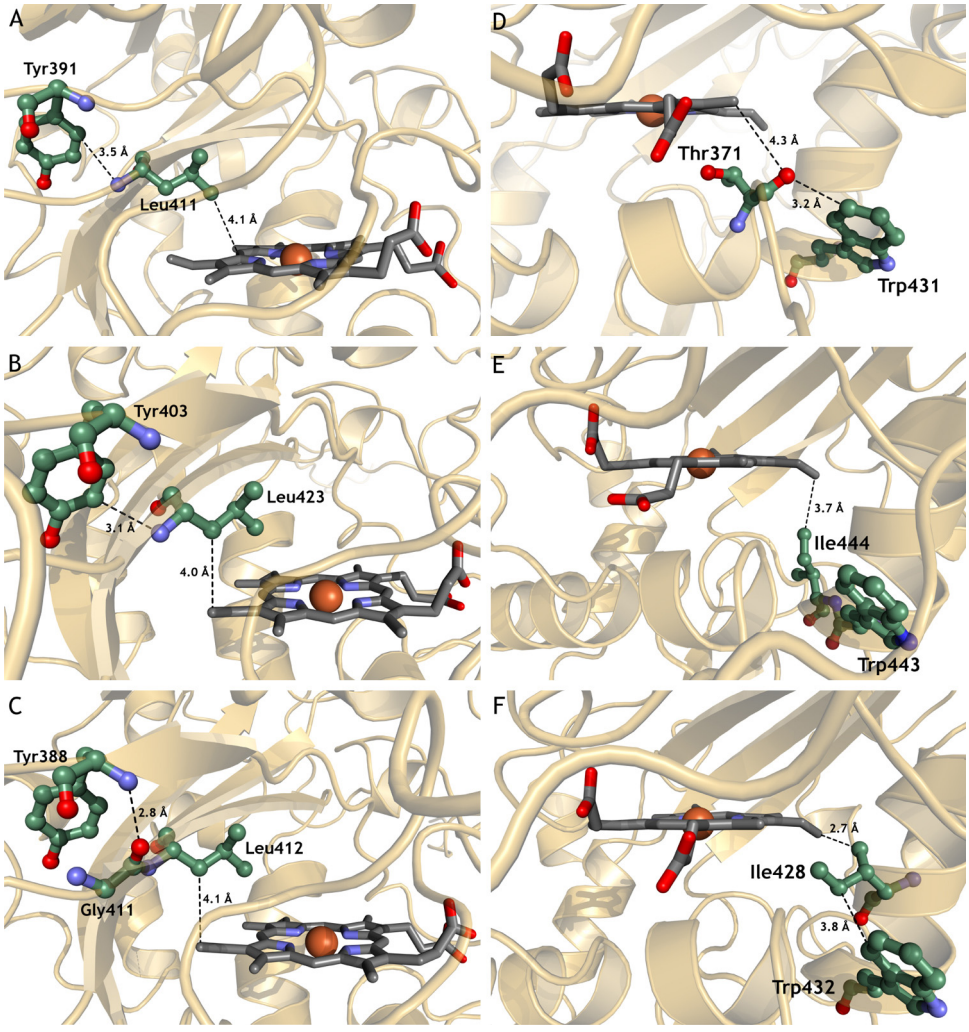

Fig. 2. Hypothetical LRET pathways in three DyP-type peroxidases, based on homology modeling: (A and D) *Egl*DyP, (B and E) *Mep*DyP, and (C and F) *Msc*DyP. The involved amino acids are depicted as ball and stick models. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Estimated redox potential values of fungal DyP-type peroxidases in comparison to phenol-oxidizing plant (SBP) and fungal (CiP) peroxidases as well as lignin peroxidase of *P. chrysosporium* (LiP).

Peroxidase	Estimated redox potential (V) Compound II/resting state
*Pch*LiP	1.26 ± 0.17
*Msc*DyP	1.20 ± 0.10
*Mep*DyP	1.19 ± 0.07
*Aau*DyP1	1.19 ± 0.04
*Aau*DyP2	1.19 ± 0.02
*Egl*DyP	1.10 ± 0.05
CiP	1.06 ± 0.07
SBP	0.93 ± 0.04

and may approve the here used method for the estimation of the redox potential of the couple compound II/native enzyme of fungal and plant peroxidases. The redox potential of all DyPs tested was found to range between 1.10 and 1.20 V, which is just slightly lower than the redox potential of LiP (1.26 V) obtained that way (Table 2).

Recent investigations on the crystal structure of *Aau*DyP1 in combination with spin-trapping experiments identified catalytically important amino acid residues that may be responsible for the LRET from the proteins surface to the redox active heme cofactor similar as it is known for LiP of *P. chrysosporium* (Trp-171 in H8, [47]) or the "untypical" LiP of *Trametopsis* (*Trametes*) *cervina* (Tyr-181 in *Tc*LiP*, [48]). A surface exposed oxidation site in *Aau*DyP1 (Tyr-337, [7]) is obviously also the key amino acid in the oxidation processes by other fungal DyPs (Tyr-391, Tyr-403 and Tyr-388 in *Egl*DyP, *Mep*DyP and r*Msc*DyP, respectively; Fig. 2A–C). The most probably electron transfer pathway ends at a Leu (Leu-411, Leu-423, Leu-412 *Egl*DyP, *Mep*DyP and r*Msc*DyP, respectively) that is, with 4.0 Å and respectively 4.1 Å, in an adequate distance to the heme-pyrrole ring and enables the reduction of the cofactor. On the other hand, there are also solvent exposed tryptophan residues (*Aau*DyP1, Trp-377 [7], (Trp-431, Trp-443 and Trp-432 in *Egl*DyP, *Mep*DyP and r*Msc*DyP, respectively; Fig. 2D–F). These residues could also act as corresponding radicals via a short LRET to the oxidized heme iron (Tyr-377 to Fe 13.9 Å, [7]). Only one amino acid is necessary for the electron transfer: threonine in case of *Egl*DyP *and Aau*DyP1 [7] or isoleucine for *Mep*DyP and r*Msc*DyP. Interestingly, almost all of these redox-active amino acid residues are conserved among the here investigated DyPs (*Aau*DyP, *Egl*DyP, *Mep*DyP, r*Msc*DyP) except for Thr-371 (*Egl*DyP) that is replaced by Ile in *Mep*DyP and r*Msc*DyP. The existence of these radical-stabilizing and electron-transferring amino acids as well as the here proved redox properties of fungal DyPs strongly indicate a mechanistic and functional similarity to high redox potential peroxidases like LiPs. Furthermore a change of conformation of Asp-168 in *Aau*DyP1 [7] from Arg to Gly after formation of compound I probably lead to a certain channel broadening and a presumable passage of small organics including phenols (Strittmatter et al. [7]). Thus it cannot be rules out that DyPs have only solvent-exposed substrate binding sites. Not least due to the secretion of DyPs under conditions close to nature (i.e. in wood cultures, up to 1.100 mU g^{-1}) they may substitute rarely found LiPs as part of the ligninolytic system of non-polyporous white-rot fungi.

4. Conclusion

Our study verified that the catalytic behavior of DyPs ranges between ligninolytic peroxidases, plant peroxidases and phenol-affine fungal peroxidases. The secretion of DyPs during fungal growth on beech wood and their ability to oxidize methoxylated aromatics (e.g. β-*O*-4 dimers and 1,2-dimethoxybenzene) indicate that they could be part of the ligninolytic system of wood-rot fungi.

Acknowledgments

Funding of this research is gratefully acknowledged to the German Environmental Foundation (DBU; project 13225-32), the European Union (integrated project BIORENEW) and to the Spanish Science and Technology Foundation (FECYT) and MICINN (grant to E. Aranda). Part of the work was supported the Hessian Ministry of Science and Art by a generous grant for the LOEWE research focus 'Insect Biotechnology'.

References

[1] K.G. Welinder, Curr. Opin. Struct. Biol. 2 (1992) 388–393.
[2] A. Hatakka, FEMS Microbiol. Lett. 13 (1994) 125–135.
[3] T.K. Kirk, R.L. Farrell, Annu. Rev. Microbiol. 41 (1987) 465–505.
[4] F.J. Ruiz-Dueñas, A.T. Martínez, in: E. Torres, M. Ayala (Eds.), Biocatalysis Based on Heme Peroxidases, Springer Verlag, Berlin, Heidelberg, 2010, pp. 37–59.
[5] M. Hofrichter, R. Ullrich, M.J. Pecyna, C. Liers, T. Lundell, Appl. Microbiol. Biotechnol. 87 (2010) 871–897.
[6] Y. Sugano, Cell. Mol. Life Sci. 66 (2009) 1387–1403.
[7] E. Strittmatter, C. Liers, R. Ullrich, S. Wachter, M. Hofrichter, D.A. Plattner, K. Piontek, J. Biol. Chem. 288 (2012) 4095–4102.
[8] C. Liers, C. Bobeth, M.J. Pecyna, R. Ullrich, M. Hofrichter, Appl. Microbiol. Biotechnol. 85 (2010) 1869–1879.
[9] M. Scheibner, B. Hulsdau, K. Zelena, M. Nimtz, L. de Boer, R.G. Berger, H. Zorn, Appl. Microbiol. Biotechnol. 77 (2008) 1241–1250.
[10] T.K. Kirk, M. Tien, P.J. Kersten, M.D. Mozuch, B. Kalyanaraman, Biochem. J. 236 (1986) 279–287.
[11] T. Lundell, R. Wever, R. Floris, P. Harvey, A. Hatakka, G. Brunow, H. Schoemaker, Eur. J. Biochem. 211 (1993) 391–402.
[12] A. Hatakka, T.K. Lundell, A.L.M. Tervilä-Wilo, G. Brunow, Appl. Microbiol. Biotechnol. 36 (1991) 270–277.
[13] I.G. Gazarian, L.M. Lagrimini, S.J. George, R.N. Thorneley, Biochem. J. 320 (1996) 369–372.
[14] J.P. McEldoon, A.R. Pokora, J.S. Dordick, Enzyme Microb. Technol. 17 (1995) 359–365.
[15] P.J. Kersten, B. Kalyanaraman, K.E. Hammel, B. Reinhammar, T.K. Kirk, Biochem. J. 268 (1990) 475–480.
[16] Z.S. Farhangrazi, M.E. Fossett, L.S. Powers, W.R. Ellis Jr., Biochemistry 34 (1995) 2866–2871.
[17] D. Dolphin, R.H. Felton, Acc. Chem. Res. 7 (1974) 26.
[18] M. Sorlie, L.C. Seefeldt, V.D. Parker, Anal. Biochem. 287 (2000) 118–125.
[19] M. Oyadomari, H. Shinohara, T. Johjima, H. Wariishi, H. Tanaka, J. Mol. Catal. B: Enzym. 21 (2003) 291–297.
[20] S.Q. Liu, H.X. Ju, Anal. Biochem. 307 (2002) 110–116.
[21] M. Ayala, R. Roman, R. Vazquez-Duhalt, Biochem. Biophys. Res. Commun. 357 (2007) 804–808.
[22] R.A. Marcus, N. Sutin, Biochim. Biophys. Acta 811 (1985) 265–322.
[23] K.E. Hammel, B. Kalyanaraman, T.K. Kirk, J. Biol. Chem. 261 (1986) 16948–16952.
[24] L.G. Fenoll, F. Garcia-Molina, M.A. Gilabert, R. Varon, P.A. Garcia-Ruiz, J. Tudela, F. Garcia-Canovas, J.N. Rodriguez-Lopez, Biol. Chem. 386 (2005) 351–360.
[25] L.K. Folkes, L.P. Candeias, FEBS Lett. 412 (1997) 305–308.
[26] F. Xu, Biochemistry 35 (1996) 7608–7614.
[27] J. Lind, X. Shen, T.E. Eriksen, G. Meréyeni, J. Am. Chem. Soc. 112 (1990) 479–482.
[28] C. Liers, M.J. Pecyna, H. Kellner, A. Worrich, H. Zorn, K.T. Steffen, M. Hofrichter, R. Ullrich, Appl. Microbiol. Biotechnol. (2012), http://dx.doi.org/10.1007/s00253-012-4521-2.
[29] K. Ikehata, I.D. Buchanan, D.W. Smith, Can. J. Microbiol. 50 (2004) 57–60.
[30] Y. Morita, H. Yamashita, B. Mikami, H. Iwamoto, S. Aibara, M. Terada, J. Minami, J. Biochem. 103 (1988) 693–699.
[31] M. Tien, T.K. Kirk, Proc. Natl. Acad. Sci. U. S. A. 81 (1984) 2280–2284.
[32] A.N. Hiner, J. Hernandez-Ruiz, J.N. Rodriguez-Lopez, F. Garcia-Canovas, N.C. Brisset, A.T. Smith, M.B. Arnao, M. Acosta, J. Biol. Chem. 277 (2002) 26879–26885.
[33] T. Schwede, J. Kopp, N. Guex, M.C. Peitsch, Nucleic Acids Res. 31 (2003) 3381–3385.
[34] A. Sali, T.L. Blundell, J. Mol. Biol. 234 (1993) 779–815.
[35] The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC.
[36] M. Zámocký, C. Obinger, in: E. Torres, M. Ayala (Eds.), Biocatalysis Based on Heme Peroxidases, Springer Verlag, Berlin, Heidelberg, 2010, pp. 7–35.
[37] C. Li, M.Z. Hoffman, J. Phys. Chem. B 103 (1999) 6653–6656.
[38] K. Ohkura, H. Hori, Bioorg. Med. Chem. 7 (1999) 309–314.
[39] H. Teramoto, H. Tanaka, H. Wariishi, Appl. Microbiol. Biotechnol. 66 (2004) 312–317.
[40] H. Wariishi, K. Valli, M.H. Gold, J. Biol. Chem. 267 (1992) 23688–52369.
[41] Z.C. Hu, R.A. Korus, C.R. Venkataramu, R.L. Crawford, Enzyme Microb. Technol. 15 (1993) 567–574.

[42] M.J. Martinez, F.J. Ruiz-Duenas, F. Guillen, A.T. Martinez, Eur. J. Biochem. 237 (1996) 424–432.
[43] A.N. Kapich, T.V. Korneichikc, A. Hatakka, K.E. Hammel, Enzyme Microb. Technol. 46 (2010) 136–140.
[44] K.A. Jensen, W. Bao, S. Kawai, E. Srebotnik, K.E. Hammel, Appl. Environ. Microbiol. 62 (1996) 3679–3686.
[45] N. Chung, S.D. Aust, Arch. Biochem. Biophys. 316 (1995) 851–855.
[46] P.G. Furtmuller, J. Arnhold, W. Jantschko, M. Zederbauer, C. Jakopitsch, C. Obinger, J. Inorg. Biochem. 99 (2005) 12209.
[47] T. Choinowski, W. Blodig, K.H. Winterhalter, K. Piontek, J. Mol. Biol. 286 (1999) 809–827.
[48] Y. Miki, H. Ichinose, H. Wariishi, FEMS Microbiol. Lett. 304 (2010) 39–46.